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## THE ACTIVITY OF COPPER-CONTAINING ENZYMES IN THE BIRCH LEAVES IN THE CONDITIONS OF THE BUILT ENVIRONMENT

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### Abstract.

In work dynamics of cupriferous enzymes in leaves of silver birch which is constant object of green construction in the anthropogenous environment is shown. Many researches indicate interrelation of adaptive opportunities of a vegetable organism and functioning of enzymatic system, including cupriferous enzymes of a poliphenoloxidaza and ascorbinatoxidaza. Activity of an ascorbinatoxidaza was determined by a method which is based on property of ascorbic acid to absorb light with a maximum at the wavelength of 265 nanometers. About activity of enzyme judged by reduction of size of optical density, considering that the oxidation level of ascorbic acid is proportional to amount of enzyme. Activity of a poliphenoloxidaza was determined by spectrotometric method which is based on measurement of optical density of products of reaction which are formed at oxidation of pyrocatechin for a certain period. During all vegetative period, plants in the conditions of the urban environment, had an increase in activity of a poliphenoloxidaza with achievement of the maximum value in August. In both years of observations (2014 and 2015) there was a decrease of the activity of an ascorbinatoxidaza in leaves during active vegetation of plants, and in the native habitat, on the contrary, its increase. The minimum values of activity of this enzyme are noted in August in the near-road plantings.

**Keywords:** silver birch, poliphenoloxidaza, ascorbinatoxidaza, anthropogenous environment, adaptation of plants

### Introduction

The modern world is characterized by continuous increase of anthropogenous impact on the environment, at the expense of an urbanization and development of industrial production. The role of plants in the anthropogenous environment consists in performance of a number of functions, of which are main environment forming, environment improving and environment regulating. In turn vegetable organisms under the influence of toxic substances I change

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biochemical, physiological and morphological properties [Galves-Valdivieso, 2010; Bukharina, 2014; Bukharina, 2014]. Many researches indicate interrelation of adaptive opportunities of a vegetable organism and functioning of enzymatic system, including cupriferous enzymes of a poliphenoloxidaza and ascorbinatoxidaza [Gill, 2010; John, 2008; Bukharina, 2015].

Poliphenoloxidaza and peroxidase in combination with phenolic substrata participate in process of breath at the intermediate stages of transfer of hydrogen. It is established that activity of a poliphenoloxidaza increases in the damaged fabrics of plants. ascorbinatoxidaza is widespread enzyme in plants which catalyzes air oxidation of ascorbic acid with formation of dehydrocarbonic acid and peroxide of hydrogen [Pachzkowska, 2007].

Research object - silver birch (*Betula pendula* Roth.), which is widespread in gardening of the city of Naberezhnye Chelny and sanitary protection zones of JSC Kamaz and differs in stability in the conditions of the technogenic environment.

The purpose of our work was studying of activity of a poliphenoloxidaza and ascorbinatoxidaza in leaves a birch of the povisly, growing in plantings various ecological categories in the large industrial center of Central Volga area.

#### **Data and methods**

Naberezhnye Chelny is a part of the Republic of Tatarstan which is located in the territory of Central Volga area. The annual amount of precipitation averages 555 mm. Average annual air temperature – 2 ... 3,1 °C. Naberezhnye Chelny – the large industrial center with the population of 530 thousand people. The key enterprise of the city is the Kama automobile plant. The characteristic of extent of pollution of atmospheric air in places of growth of wood plants is carried out by us on the basis of materials of "The report on an ecological condition of the Republic of Tatarstan" for 2015. The complex index of pollution of the atmosphere (IZA=15,3) characterizes state of pollution of atmospheric air in the city as very high. Excess of level of threshold limit value on gas is established (and) to pyrene, formaldehyde, phenols, carbon oxides and nitrogen. In the sanitary protection zone of the industrial enterprises average annual excess of maximum allowable concentration is noted on the following substances: carbon oxide – twice; nitrogen oxides – by 3 times; sulfur dioxide – by 1,2 times; formaldehyde – by 5 times; phenol – by 1,7 times; gas (and) pyrene – by 1,9 times. In a zone of the main landings average annual excess of maximum allowable concentration is noted on the following substances: carbon oxide – by 3,4 times; formaldehyde – by 3,8 times; phenol – by 1,4 times; gas (and) pyrene – by 1,5 times [State ..., 2015].

Research object - silver birch (*Betula pendula*) grows in the city as a part of plantings of different ecological categories: main landings (large highways of the Car 1 and Mira Avenue); plantings of the sanitary protection zones (SPZ) of the industrial enterprises of JSC Kamaz, the Liteyny and Kuznechny plants which are the main pollutants of the city. As the zone of conditional control (ZCC) the territory of the Naberezhnye Chelny forest area of 9539 hectares (the forest-steppe region of the European part of the Russian Federation) is chosen.

The Trial Areas (TA) not less than 0,25 hectares in size put in the regular way (5 software in each planting).

Within the trial areas for studying of physiology-biochemical indicators of silver birch selection, numbering and assessment of a vital state not less than 10 individuals were made. Registration individuals had a good vital shape and a middle-aged generative ontogenetic state (g2). During active vegetation, i.e. June, July and August at registration individuals made sampling of leaves of a median formation on year vegetative escape (from the lower third of the site of krone of the southern exposition). Leaves of a median formation – the leaves, typical for a plant, developing in an average zone of escape and performing photosynthesis function [Korovkin, 2007]. Within software selection of soil tests (the mixed test made of individually taken samples on a way of an envelope) was made. In the main plantings a part of krone of the southern exposition was turned directly to the carriageway of the prospectus.

In vitro determined activity of an ascorbinatoxidaza by the method offered by D. K. Asamov, S. T. Rakhimova [Ermakov, etc., 1987] which is based on property of ascorbic acid to absorb light with a maximum at the wavelength of 265 nanometers. About activity of enzyme judged by reduction of size of optical density, considering that the oxidation level of ascorbic acid is proportional to amount of enzyme. Activity of a poliphenoloxidaza was determined by spectrotometric method which is based on measurement of optical density of products of reaction which are formed at oxidation of pyrocatechin for a certain period. For each individual analyses carried out in three times. Processing of materials was carried out with application of a statistical package "Statistica 5.5". For the analysis of the obtained data used methods of descriptive statistics and the dispersive multiple-factor analysis, with the subsequent assessment of distinctions by method of multiple comparison (LSD-test).

## **Results and Discussion**

In 2014 the vegetative period was characterized by the increased air temperature, excess of mean annual values of air temperature was within +3 ... +7°C, at the same time rainfall was within norm. In 2015 conditions were less droughty, than in 2014: excess of air temperature over mean annual values made +1 ... +2°C, and loss of rainfall exceeded norm.

The carried-out agrochemical analysis showed that soils in plantings of zones of conditional control had alkaline reaction ( $\text{pH}_{\text{KCl}} = 7.1\text{--}7.2$ ). Content of organic substance was at the average level (5.3–5.8%), the content of mobile phosphorus varied from raised to very high (115.4–291.3 mg/kg), exchange potassium – from high to very high (210–314 mg/kg). In soils the high content of nitrate forms of nitrogen (210–405) and low contents the ammonium forms of nitrogen (8.3–19.3 mg/kg) was noted.

In plantings of sanitary protection zones of the industrial enterprises of the soil were characterized by subacidic and alkaline reaction ( $\text{pH}_{\text{KCl}} = 6.7\text{--}7.5$ ). Content of organic substance varied from an average to raised (5.5–6.2%). The maintenance of nitrate forms of nitrogen was at the level of 247–300 mg/kg, and the ammonium forms of nitrogen – at the level of 6.1–14.9 mg/kg.

In the main plantings of the soil had exchange acidity 7.4–7.7 ( $\text{pH}_{\text{KCl}} = 8.4\text{--}8.6$ ), the alkaline reaction, and low content of organic substance (1.7–3.1%) reflecting them. Content of ammonium nitrogen (6.4–8.1 mg/kg) and mobile phosphorus ( $\text{P}_2\text{O}_5 = 29.8\text{--}53.5$  mg/kg) varied from low to an average. Content of nitrate nitrogen (164–175 mg/kg) was at the average level, and exchange potassium ( $\text{K}_2\text{O} = 210\text{--}325$  mg/kg) – from high to very high.

The dispersive multiple-factor analysis of results of researches in 2014 and 2015 revealed reliability of influence of a complex of conditions of the place of growth (significance value  $P \leq 10^{-5}$ ), phases of the vegetative period ( $P \leq 10^{-5}$ ), years of growth ( $P \leq 10^{-5}$ ), and also interactions of these factors ( $P = 5,3 \cdot 10^{-5}$ ) on activity of a polyphenoloxidase in leaves of silver birch (tab. 1).

**Activity of a polyphenoloxidase in leaves of silver birch in the conditions of the technogenic environment, unit the act.**

Stand type	Month	Research years		Mean value
		2014	2015	
Zone of conditional control	June	1,29	1,47	1,38
	July	3,53	3,51	3,52
	August	1,82	2,88	2,35
Sanitary buffer	June	1,14	1,92	1,53
	July	2,51	3,56	3,04
	August	3,89	5,10	4,50
Main landings	June	1,21	2,26	1,74
	July	2,50	3,58	3,04
	August	3,99	5,58	4,78
$\text{HCP}_{05}$		0,02	0,04	0,04

During the entire period of active vegetation, the individuals growing in the conditions of the urban environment had an increase in activity of a polyphenoloxidase with achievement of the maximum value in August.

At the same time dynamics of activity of enzyme was various in days of a research. In 2015 it is not dependent on a zone of growth and the period of vegetation of value of activity of a poliphenoloxidaza were higher, than in 2014. So in June in 2014 in a zone of conditional control of an individual of silver birch had activity of enzyme 0,15 higher and 0,08 pieces the act., in comparison with individuals in the sanitary protection zone and the main plantings, respectively. In 2015 for the same period we observed increase in activity of enzyme at plants in city plantings on 0,45 units the act. in the sanitary protection zone and on 0,79 units the act. in plantings of the main landings. Further in 2014 in July activity of a poliphenoloxidaza at plants in control was 1,02 higher also than 1,03 units the act., than, respectively, in the SPZ of the industrial enterprises and the main plantings. In August the picture changed also values of activity of a poliphenoloxidaza in leaves of silver birch in city plantings were 2,07 - 2,17 pieces higher the act. in comparison with a zone of conditional control.

In 2015 in July and August at plants in plantings of the SPZ of the industrial enterprises and the main landings activity of a poliphenoloxidaza was 0,05 - 0,08 higher and than 2,24 - 2,70 units the act., respectively, than in control. Increase in activity of a poliphenoloxidaza, apparently, is a peculiar reaction of cages to increase of the need for breath caused by the high content of the dust particles and other pollutant interfering normal gas exchange of leaves of plants. Considering that high activity of enzyme remains also at the final stages of active vegetation of plants, it is possible to assume that there are also more rough mechanical damages of fabrics and cages of leaves.

The dispersive multiple-factor analysis of results of researches in 2014 and 2015 revealed reliability of influence of a complex of conditions of the place of growth ( $P < 10^{-5}$ ), phases of the vegetative period ( $P = 8,15 \cdot 10^{-5}$ ), years of growth ( $P = 2,88 \cdot 10^{-5}$ ), and also interactions of these factors ( $P = 1,61 \cdot 10^{-5}$ ) on activity of an ascorbinatoxidaza in leaves of silver birch (tab. 2).

**Activity of an ascorbinatoxidaza in leaves of silver birch in the conditions of the technogenic environment, unit the act.**

Stand type	Month	Research years		Mean value
		2014	2015	
Zone of conditional control	June	3,05	2,81	2,93
	July	3,40	3,34	3,37
	August	5,29	4,63	4,96
Sanitary buffer	June	2,80	2,47	2,64
	July	2,42	2,86	2,64
	August	1,91	2,38	2,14

<b>Main landings</b>	<b>June</b>	<b>2,82</b>	<b>2,55</b>	<b>2,68</b>
	<b>July</b>	<b>2,16</b>	<b>2,34</b>	<b>2,25</b>
	<b>August</b>	<b>1,37</b>	<b>2,03</b>	<b>1,70</b>
<b>HCP<sub>05</sub></b>		<b>0,03</b>	<b>0,04</b>	<b>0,04</b>

On average for 2014 and 2015 plants in city plantings had a decrease of the activity of an ascorbinatoxidaza for the entire period of active vegetation, and in the native habitat (control plantings), on the contrary, its increase. The smallest values of activity of this enzyme are noted in Auguste in the near-road plantings.

Dynamics of activity of an ascorbinatoxidaza differed by years of a research. In the conditions of the technogenic environment in 2015 activity of enzyme was higher in July and August on 0,44 and 0,47 in plantings of the SPZ of the industrial enterprises and on 0,18 and 0,66 units the act. in the near-road landings, respectively, in comparison with the same periods of previous year. Decrease of the activity of an ascorbinatoxidaza in leaves of plants in city landings, most likely, is connected with participation of ascorbic acid in system of antioxidant protection of plants in the conditions of influence of sour industrial gases.

## Conclusion

During the entire period of active vegetation, individuals of silver birch, growing in the conditions of an anthropogenous stress, had an increase in activity of a poliphenoloxidaza with achievement of the maximum value in Auguste. Increase in activity of a poliphenoloxidaza, it is possible to explain with influence of the hypoxemic stress caused by violation of gas exchange of leaves in the city conditions differing high the maintenance of the weighed particles and the gaseous pollyutant settling on a surface of leaves. Considering that high activity of enzyme remains also at the final stages of active vegetation, it is possible to believe that plants have also more rough mechanical damages of fabrics and cages.

Decrease of the activity of an ascorbinatoxidaza in leaves of plants in the urban environment, in our opinion, can be connected with an antioxidant role of ascorbic acid.

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